

Complete Genome Sequence of “*Vulcanisaeta moutnovskia*” Strain 768-28, a Novel Member of the Hyperthermophilic Crenarchaeal Genus *Vulcanisaeta*[▽]

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Strain 768-28 was isolated from a hot spring in Kamchatka, Russia, and represents a novel member of the *Vulcanisaeta* genus. The complete genome sequence of this thermoacidophilic anaerobic crenarchaeon reveals genes for protein and carbohydrate-active enzymes, the Embden-Meyerhof and Entner-Doudoroff pathways for glucose metabolism, the tricarboxylic acid cycle, beta-oxidation of fatty acids, and sulfate reduction.

Together with the genera *Thermoproteus*, *Pyrobaculum*, *Thermocladium*, *Caldivirga*, and *Thermofilum*, the genus *Vulcanisaeta* forms the crenarchaeal order *Thermoproteales* comprising rod-shaped thermophilic crenarchaeotes (5). Two currently known *Vulcanisaeta* species, *V. distributa* and *V. souniana*, are heterotrophic, anaerobic hyperthermophiles isolated from hot springs in Japan (3). The complete genome sequence of *V. distributa* DSM 14429 was determined recently (7). Strain 768-28 was isolated from the solfataric field close to Moutnovsky volcano in Kamchatka, Russia, and was found to be affiliated with the *Vulcanisaeta* genus based on 16S rRNA gene phylogeny (8). *Vulcanisaeta* sp. 768-28 is an obligately anaerobic acidophile with a pH range of growth from 3.5 to 6.5 and a temperature range from 60 to 98°C. It is a metabolically versatile archaeon capable of fermenting proteinaceous substrates and some sugars (8). Elemental sulfur and thiosulfate are reduced to hydrogen sulfide if added (8). To understand the physiological properties of this archaeon and possible ecological roles of *Vulcanisaeta* sp., a complete genome sequence was determined.

The genome of *Vulcanisaeta* sp. 768-28 was sequenced with the Roche 454 GS FLX pyrosequencing platform. We obtained two libraries of 174,571 single-strand reads and 72,003 paired-end reads containing 3-kb inserts. The reads were assembled into a single scaffold (14 contigs) by the Newbler Assembler 1.1 (454 Life Sciences, Branford, CT). The genome was finished by filling gaps with sequencing and primer walking of PCR products with an ABI 3730 capillary sequencer (Applied Biosystems, CA).

The complete genome of *Vulcanisaeta* sp. 768-28 consists of 2,298,983 bp in a single circular chromosome with an

average G+C content of 42.4%. A total of 2,320 protein-coding genes were predicted by Glimmer (1), covering 88% of the chromosome. About 77% of these proteins showed similarity to those of *V. distributa* DSM 14429, while remaining 537 proteins are specific for strain 768-28, indicating that this strain may be classified as a novel species, “*Vulcanisaeta moutnovskia*.” A whole-genome annotation and analysis were performed with the AutoFACT tool (6), followed by a round of manual curation. Metabolic pathway analysis revealed that utilization of polysaccharides and proteins involve the function of numerous encoded hydrolytic enzymes. Similarly to *Thermoproteus tenax* (10), further oxidation of monomers proceeds in both the modified Embden-Meyerhof and Entner-Doudoroff pathways followed by an oxidative tricarboxylic acid cycle enabling the complete oxidation of organic substrates to CO₂ and H₂S (9). The genome encodes at least 14 putative esterases and a complete beta-oxidation pathway for fatty acids, suggesting the ability of *V. moutnovskia* to utilize lipids. The genome analysis suggests the presence of a sulfate reduction pathway that is quite unusual among archaea and to date had been reported only in the euryarchaeon *Archaeoglobus fulgidus* (4) and crenarchaeon *Caldivirga maquilingensis* (2). Making the genome sequence of *V. moutnovskia* 768-28 available will allow comprehensive comparisons with other members of the genus and enable further investigation into the mechanisms of adaptation to thermal acidic environments.

Nucleotide sequence accession number. The complete genome sequence of *V. moutnovskia* 768-28 was deposited in GenBank under accession no. CP002529.

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REFERENCES

1. Delcher, A. L., D. Harmon, S. Kasif, O. White, and S. L. Salzberg. 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* 27:4636–4641.
2. Itoh, T., K. Suzuki, P. C. Sanchez, and T. Nakase. 1999. *Caldivirga maquilingensis* gen. nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. *Int. J. Syst. Bacteriol.* 49:1157–1163.

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3. Itoh, T., K. Suzuki, and T. Nakase. 2002. *Vulcanisaeta distributa* gen. nov., sp. nov., and *Vulcanisaeta souniana* sp. nov., novel hyperthermophilic, rod-shaped crenarchaeotes isolated from hot springs in Japan. *Int. J. Syst. Evol. Microbiol.* **52**:1097–1104.
4. Klenk, H. P., et al. 1997. The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature* **390**:364–370.
5. Kletzin, A. 2007. General characteristics and important model organisms, p. 14–92. In R. Cavicchioli (ed.), *Archaea: molecular and cellular biology*, ASM Press, Washington, DC.
6. Koski, L. B., M. W. Gray, B. F. Langi, and G. Burger. 2005. AutoFACT: an automatic functional annotation and classification tool. *BMC Bioinformatics* **6**:151.
7. Mavromatis, K., et al. 2010. Complete genome sequence of *Vulcanisaeta distributa* type strain (IC-017). *Stand. Genomic Sci.* **3**:117–125.
8. Prokofeva, M., et al. 2005. Cultivated anaerobic acidophilic/acidotolerant thermophiles from terrestrial and deep-sea hydrothermal habitats. *Extremophiles* **9**:437–448.
9. Selig, M., and P. Schönheit. 1994. Oxidation of organic compounds to CO₂ with sulfur or thiosulfate as electron acceptor in the anaerobic hyperthermophilic archaea *Thermoproteus tenax* and *Pyrobaculum islandicum* proceeds via the citric acid cycle. *Arch. Microbiol.* **162**:286–294.
10. Zaparty, M., B. Tjaden, R. Hensel, and B. Siebers. 2008. The central carbohydrate metabolism of the hyperthermophilic crenarchaeote *Thermoproteus tenax*: pathways and insights into their regulation. *Arch. Microbiol.* **190**:231–245.